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Effects of Salinity, Temperature and Food Type on the Uptake and Elimination Rates of Cd, Cr, and Zn in the Asiatic Clam *Corbicula fluminea*

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Abstract – Laboratory radiotracer experiments were conducted to determine assimilation efficiencies (AE) from ingested algal food and oxic sediment particles, uptake rates from the dissolved phase, and the efflux rates of Cd, Cr and Zn in the Asiatic clam *Corbicula fluminea*. Among three elements, AE from both algal and sediment food was greatest for Cd, followed by Zn and Cr. The AEs of tested elements from algal food (*Phaeodactylum tricornutum*) were consistently higher than those from sediments at a given salinity and temperature. The influence of salinity (0, 4 and 8 psu) and temperature (5, 13 and 21ºC) on the metal AEs was not evident for most tested elements, except Cd AEs from sediment. The rate constant of metal uptake from the dissolved phase (k_u) was greatest for Cd, followed by Zn and Cr in freshwater media. However, in saline water, the k_u of Zn were greater than those of Cd. The influx rate of all tested metals increased with temperature. The efflux rate constant was greatest for Cr (0.02 d^{-1}), followed by Zn (0.010~0.017 d^{-1}) and Cd (0.006 d^{-1}). The efflux rate constant for Zn in clam tissues depurated in 0 psu (0.017 d^{-1}) was faster than that in 8 psu (0.010 d^{-1}). Overall results showed that the variation of salinity and temperature in estuarine systems can considerably influence the metal bioaccumulation potential in the estuarine clam *C. fluminea*. The relatively high Cd accumulation capacity of *C. fluminea* characterized by the high AE, high dissolved influx rate and low efflux rate, suggested that this clam species can be used as an efficient biomonitor for the Cd contamination in freshwater and estuarine environments.

Key words – *Corbicula fluminea*, radiotracer, bioaccumulation, uptake, elimination, salinity, temperature, metal

1. Introduction

Bivalves are an important component of the benthos in estuarine and coastal ecosystems and are commonly used as biomonitors to assess exposure of aquatic organisms to metal contaminants (Goldberg *et al*. 1983). Bivalves can accumulate metals from both dissolved phases and facilitate ingestion of particulate material. Recently, studies concerning the ecological risks posed by metal pollution in aquatic environments have focused on the multi-pathway bioaccumulation modeling, which includes both uptake routes (Luoma *et al.* 1992; Wang *et al.* 1996; Lee *et al.* 2001).

In estuarine and coastal environments, various environmental factors including salinity, temperature, and food availability can vary widely. Those external factors were known to influence on the metal bioaccumulation in organisms by changing either the bioavailability of dissolved and particulate metals in water or physiological attributes of organisms. However, few bioaccumulation or biokinetic studies have concerned the influence of varying environmental factors. Therefore, the comparative influences of many of those factors are not fully understood. Quantitative understanding of how the environmental factors affect metal bioaccumulation is critical to accurately assess the dose of metal that animals in the field will experience. Therefore, it would be a prerequisite for modeling bioaccumulation and also for the use of biomonitors in environmental monitoring programs.

Ambient temperature can influence the metabolic rate of poikilotherms. Therefore, metal uptake from water and food in bivalves can be influenced by the variation in water temperature. Salinity can also influence metal bioavailability by changing the geochemistry of metals and physiological attributes of aquatic organisms. However, few bioaccumulation or biokinetic studies have evaluated the influence of temperature and salinity on the uptake of metals. In particular, the metal uptake and retention from dietary uptake under varying external conditions is largely unknown (Croteau *et al*. 2002).

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Relatively less attention has been paid to the influence of food assemblages on metal uptake by aquatic organisms, while most studies have been concerned with the metal uptake from the dissolved phase of metals (Lee and Luoma 1998; Lee *et al.* 2000). Some studies suggested that metals associated with the living particles might be relatively more bioavailable via ingestion compared to metals in inorganic particles (Reinfelder and Fisher 1991) probably due to the digestive strategy of organisms, which may retain nutritious food for a longer time (Decho and Luoma 1991). Some previous studies proposed that the gut retention time of metals associated with particles could be functionally related to the assimilation efficiency of metals. However, the empirical relationship between gut retention time and the assimilation efficiency of ingested metals should be systematically evaluated under various experimental conditions.

Both water ventilation and food ingestion are important metal uptake routes for filter-feeding bivalves. Therefore, several physiological parameters can influence the metal bioaccumulation, such as ingestion rate, assimilation efficiency of metals in the intestine, clearance rate, metal absorption efficiency in gill, and turnover rate of the absorbed metals in tissues (Wang *et al*. 1996). Among these factors, assimilation efficiency, dissolved metal uptake rate and efflux rate of absorbed metal are kinetically determined using radiotracers. Radiotracer approaches can reduce the enormous biological replication and huge experimental setup because one can determine metal concentration in test organisms non-destructively.

Asiatic clams *Corbicula fluminea* were adopted herein to investigate the influence of salinity and temperature, the most important environmental factors in the estuarine system, on the influx and efflux of various metals. Edible clams *C. fluminea* inhabited worldwide in estuaries as well as freshwater systems. Although many *C. fluminea* were found in brackish waters in San Francisco Bay and also in other areas including Korea, most previous ecotoxicological studies using *C. fluminea* were conducted only in freshwater conditions (*e.g.* Graney *et al.* 1983; Inza *et al.* 1998).

In this study, we quantified the various physiological parameters characterizing the metal bioaccumulation from particulate and dissolved uptake by *C. fluminea* under varying temperature and salinity conditions. To determine assimilation efficiencies of Cd, Cr and Zn from the ingested particles, clams were fed different types of radiolabeled particles. To quantify the influx rate of dissolved Cd, Cr and Zn, clams were exposed to radiolabeled water media having a range of metal concentration under different salinity and temperature conditions. Efflux rates of Cd, Cr and Zn were also determined after clams were exposed to radiolabeled water and food in both freshwater and saltwater media.

2. Materials and Methods

Test animals

The Asiatic clam *Corbicula fluminea* of similar size (0.19±0.01 g dry wt.) were collected from a site in freshwater stream (0 psu, 12°C) located in San Jose, CA, USA, about 1 week prior to each experiment. Upon returning to the laboratory, the clams were gradually acclimated for a week to the experimental salinity (0, 4 or 8 psu) and temperature (5, 13 or 21°C) levels, respectively. The salinity and temperature of water media for clam acclimation were changed by <2 psu and 2°C per day, if it was considered necessary. During the acclimation, clams were fed a mixed food of diatom algae (*Phaeodactylum tricornutum*) and <64-μm surface sediment from the same collection site of *C. fluminea*.

Freshwater water media (0 psu) was prepared artificially using a Fraquil recipe (Morel *et al.* 1975), and saline media (4 and 8 psu) were made by diluting 0.2-μm of filtered natural seawater (35 psu, collected from Biological Laboratory, UC Santa Cruse) with the freshwater media in adequate ratios. The clams were not fed for 2 d prior to any experiment to encourage the ingestion of particles by animals during the feeding experiment.

Assimilation efficiency of ingested particles

The pulse-chase method was used to determine assimilation efficiencies (AE) of Cd, Cr and Zn from ingested radiolabeled sediment and algae *P. tricornutum*, followed by an established protocol (Lee and Luoma 1998). Effects of salinity and temperature on the AE of metals were also evaluated in the experimental solution at various temperature and salinity levels. The effect of salinity (0, 4 and 8 psu) was evaluated only at 13°C and that of temperature (5, 13 and 21°C) was evaluated only in freshwater media (0 psu).

Radiolabeled algae was prepared by culturing diatom species *Phaeodactylum tricornutum* in 5 μCi of ^{109}Cd-, 12 μCi of ^{51}Cr(VI)-, and 3 μCi of ^{65}Zn-spiked seawater media (15 psu) enriched with f/2 nutrients (N, P, Si, vitamins, Fe without EDTA, trace metals) at 20°C for 10 days. A periodical light-dark cycle (14h:10h) was used for algal cultures. The initial cell density was 1×10^4 cells/ml and was increased to 6×10^6 cells/ml during the incubation process. Experimental sediment was collected from the natural stream and the sediment particles between 1~63 μm were sorted by sieving and settling methods (Folk 1954). Radiolabeled sediment was also prepared at 20°C by equilibrating oxidized sediment with 7 μCi of ^{109}Cd-, 10 μCi of ^{51}Cr(VI)-, and 5 μCi of ^{65}Zn dissolved in a solution having 3 different salinity levels (0, 4 and 8 psu) for 10 days.

Following the radiolabeling procedure of algae and sediment, the radiolabeled particles were filtered with 0.45-μm polycarbonate membrane and resuspended in unlabeled water media of 5 different salinity-temperature

combinations (0-13, 4-13, 8-13, 0-5 and 0-21; psu-°C) to yield a cell density of ~5×10^4 cells/ml. Resuspended radiolabeled algal and sediment particles were used as food in the assimilation efficiency experiment. Following the acclimation to each experimental salinity and temperature level, *C. fluminea* were divided into 12 groups (2 or 4 food type ×5 salinity-temperature pairs) of 30 individuals. Each group was placed in a feeding chamber with 1 L of water media equilibrated to the experimental temperature level for 24 h.

During the pulse-chase feeding period, *C. fluminea* were exposed to radioactive particles for 1.5 hr (2 hr for clams at 5°C), so that clams ingested a measurable amount of radioactivity. Following the pulse feeding, clams were held in uncontaminated water media for 10 min (short depuration) to remove or reduce pseudofeces, radioactive water contained in the cavity of clams, and adsorbed metals on shells. Following the short depuration, four clams fed radiolabeled particles were dissected to determine the ratio of radioactivity between tissue and shell and remaining clams were placed in the long-term depuration chambers.

Clams fed radiolabeled particles were depurated for 48 hr. During the depuration, unlabeled water was changed periodically. Whole body activity remaining in the clams and egested radioactivity in feces were determined at t = 0, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 h. During this procedure, animals were nondestructively counted for radioactivity and returned to the cold feeding chamber for continuous depuration. Following the 48-h depuration, clams were dissected to determine the ratio of radioactivity in the soft tissue and shells, which can vary according to the depuration period and experimental conditions. Assimilation efficiency (AE) was calculated by establish protocols (Luoma *et al.* 1992; Lee and Luoma 1998). The GPT was defined operationally as the time at which 90% of the cumulative defecation of an element is recovered (Wang *et al.* 1995), assuming 100% recovery at 48 h in this study.

Influx rate from the dissolved phase

To determine the effect of metal concentration on the influx rate, *C. fluminea* were exposed to a range of Cd (0.1~10 μg/L), Cr (0.3~30 μg/L), and Zn concentrations (0.5~50 μg/L). An experimental medium was prepared by the addition of Cd and Zn AAS standards, in diluted nitric acid, and $Na_2CrO_4{\cdot}4H_2O$ to 2 L of 0.2-μm filtered water media.

To evaluate the influence of salinity and temperature on the influx of metals, water media having 3 different salinities (0, 4, and 8 psu) at 13°C or 3 different temperatures (5, 13, 21°C) at 0 psu were prepared. Radioactive metals were used as tracers of stable metals; each container received 1 μCi of ^{109}Cd, 2 μCi of ^{51}Cr(VI), and 1 μCi of ^{65}Zn. Adequate amounts of 0.1M NaOH was added to adjust the pH level of water media in each container to 8.

Fifteen individual clams per treatment were exposed to stable and radiolabeled metals in water media. Clams were exposed to radioactive media for 6 h at designed salinities (0, 4 and 8 psu) and temperatures (5, 13 and 21°C). Radioactivity in experimental media was monitored periodically by sampling 2 ml of the exposure media. Following the metal exposure, clams were removed from the media, rinsed with unlabeled water and randomly grouped with 3 individuals per group. Clams were then dissected and the radioactivity in the soft tissue and shell was assayed separately. Soft tissue and shells were dried at 70°C and weighed separately.

The effects of metal concentration on influx rates of Cd, Cr and Zn from the dissolved phase can be expressed by the following equation, $I_w = k_u C_w^{\,b}$, where I_w is metal influx rate to clam tissue (μg metal g^{-1} [dry wt] d^{-1}), k_u is the dissolved metal uptake rate constant ($L{\cdot}g^{-1}{\cdot}d^{-1}$), C_w is the metal concentration in water and b (power coefficient) is the slope of the log-log relationship between I_w and C_w. If the b is close to 1, k_u can be calculated easily from the above equation using the first-order modified equation, $I_w = k_u C_w$.

Efflux rate of absorbed metals

The efflux rates of metals were determined from ~80 clams exposed to both metal-contaminated food (algae and sediment) and solution for 6 d and depurated for 21 d in unlabeled freshwater (0 psu) or saline water media (8 psu) at 13°C. The 3 random-sized clams were grouped (total 21 groups) and each group was immediately assayed for whole body radioactivity (t = –2 d). The clams were depurated initially for 2 d to remove the unassimilated particles. Following the 2-d of the defecation period, three groups were sacrificed to estimate the partitioning of radioactivity between the soft tissue and shell at t = 0 d. The remaining groups were transferred to depuration chambers filled with seawater recirculated through the activated charcoal filter to remove metals from the solution.

Clams were fed on unlabeled algae and allowed to depurate the accumulated metals for an additional 19 d. During the depuration periods, each group was temporarily removed from the depuration chamber to measure whole body radioactivity. A group was sacrificed at t = 1, 3, 7, 11 and 16 d to determine the proportion of radioactivity partitioned to the soft tissue and shell. Following the 19 d depuration, the remaining 13 groups were measured for total radioactivity and, following dissection, re-assayed for radioactivity in tissue and shell. The temporal changes in the radioactivity of soft tissue for the clams were estimated by multiplying whole clam radioactivity by soft tissue/shell ratios obtained from clams that were dissected periodically. Clam tissues were then dried to a constant

weight at 70°C.

The physiological turnover of assimilated Cd in clam tissue followed 1-compartmental a first order exponential function, $R_t = R_0 e^{-k_e t}$, where R_t is the fraction of metal retained in clam tissue at Day t and k_e is the rate constant of loss, and t is time (d).

Radioactivity and data analysis

Radioactivity was determined with a gamma counter equipped with a 3-inch well-type NaI crystal detector. Photon emissions of ^{109}Cd were determined at 88 keV, ^{51}Cr at 320 keV, and ^{65}Zn at 1,115 keV. The counting times for all samples were 2~5 min, and propagated counting errors were generally <5%. All the dpm values were corrected for decay and expressed in terms of unit dry weight of soft tissue. Statistical significance was set at $\alpha = 0.05$, otherwise noted. Dry weight based concentrations were used for all tissue data. Statistica® (StatSoft Inc., USA) was used for statistical analyses including regression analysis.

3. Results

Retention of ingested Cd, Cr and Zn in clam tissues

Corbicula fluminea defecated most metals during the initial 8-16 h of depuration (Fig. 1). The range of 70~95% of the ingested metals were egested within 16 h after the pulse feeding. To compare the egestion rate among different elements, food compositions, and external conditions (salinity and temperature), the gut passage time (GPT) of ingested metals in clams was calculated for all food types and all elements (Table 1).

Food composition might influence the retention of metals in clam guts, especially in freshwater media (Fig. 1). Metals associated with algal food are retained longer than those bound to sediment only at 0 psu (Table 1). However, the influence of food type on the GPT of metals was mitigated in saline media (4 and 8 psu).

The GPT of all elements associated in the case of both algae and sediment significantly decreased with the increase of salinity (Fig. 1; Table 1). There was no considerable difference in GPTs of ingested metals among food types in saltwater media. The effect of temperature on GPT was not consistent in terms of different elements and/or food types (Table 1).

Assimilation efficiency of ingested metals

The AEs of Cd by *C. fluminea* (50~90%) was greatest, followed by Zn AEs (23~63%) and Cr AEs (2~5%) over

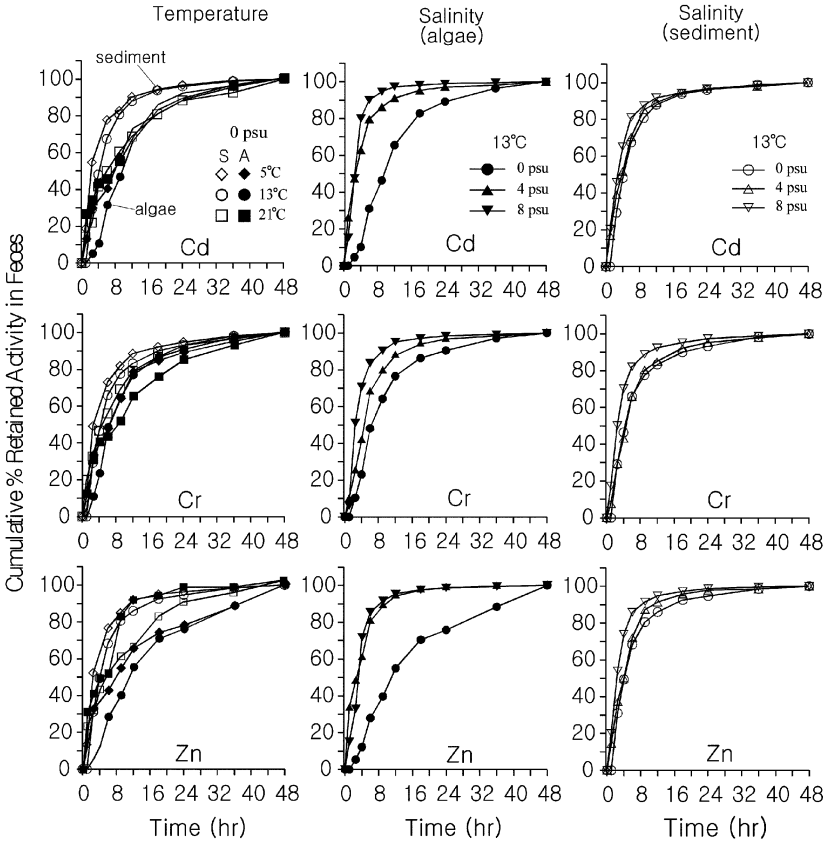

Fig. 1. Cumulative relative activity (%) of egested radioactive metals in feces of *Corbicula fluminea* ingesting algal or sediment food under varying salinity levels (0, 4, 8 psu) at 13°C or at varying temperatures (5, 13, 21°C) at 0 psu.

Table 1. Gut passage time (hr) of ingested metals in *Corbicula fluminea*.

Temp. (°C)	Salinity (psu)	Algae			Sediment		
		Cd	Cr	Zn	Cd	Cr	Zn
13	0	25.3	23.3	37.7	14.1	18.0	15.7
13	4	11.3	14.1	9.7	13.0	16.2	11.0
13	8	6.0	8.8	8.2	10.1	9.9	8.2
5	0	27.0	26.1	37.8	11.9	14.2	11.2
13	0	25.3	23.3	37.7	14.1	18.0	15.7
21	0	22.2	31.6	12.7	28.6	21.1	27.4

Table 2. Assimilation efficiency (%) of Cd, Cr and Zn from algae and sediment into *Corbicula fluminea*.

Temp. (°C)	Salinity (psu)	Algae			Sediment		
		Cd	Cr	Zn	Cd	Cr	Zn
13	0	90	7.5	74	55	1.9	29
13	4	88	7.7	81	35	1.5	31
13	8	79	7.4	69	47	1.7	37
5	0	83	4.7	75	39	1.4	25
13	0	90	7.5	74	55	1.9	29
21	0	89	4.5	65	84	1.2	19

the range of food type, salinity, and temperature. The food type significantly influenced on the AEs of all elements. The AEs of Cd, Cr and Zn associated with algal food were 2~3 times higher than those with sediment food (Table 2).

The influence of salinity and temperature on the AEs was observed only for Cd. The salinity significantly varied the AEs of Cd in both algae and sediment food and the AE of Cd associated with sediment sharply increased as water temperature increased (Table 2).

The significant relationship between the gut passage time and AE of ingested Cd and Cr were shown in freshwater media (Fig. 2). In spite of the reduced GPT, the AEs of metals in algae from saline media were comparable to those that had undergone freshwater exposure (Fig. 2).

Influx rate from the dissolved phase

The metal influx rates increased linearly ($p<0.001$) with the exposure concentration (Fig. 3). There had little concentration effect on the dissolved Cd and Cr uptake; the slopes (*b*) for the Cd and Cr uptake were close to 1 (Fig. 3; Table 3). However, most slopes for the Zn uptake were less than 1 (0.5–0.8). The concentration effect of Zn might disappear at the lowest temperature (5°C) at which dissolved uptake of Zn was lowest (Table 3).

The influx rate constant (k_u) of Cd by clams was greatest, followed by Zn and Cr in freshwater media, while that of Zn was greatest in saline media (Table 3). The higher was water temperature, the faster was the uptake

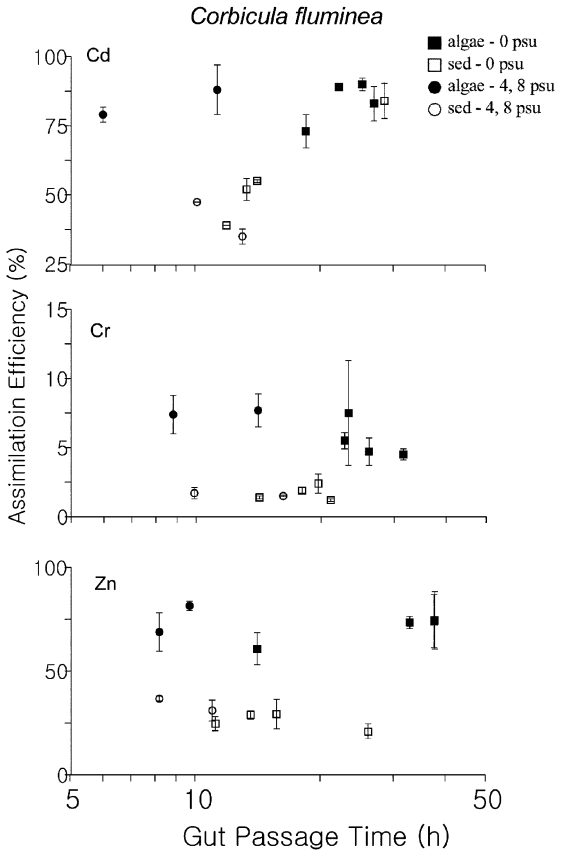

Fig. 2. Relationships of metal assimilation efficiencies (AEs; %) with gut passage time (GPT; hr) of Cd, Cr and Zn in *Corbicula fluminea* fed algal or sediment particles under varying salinity and temperature levels.

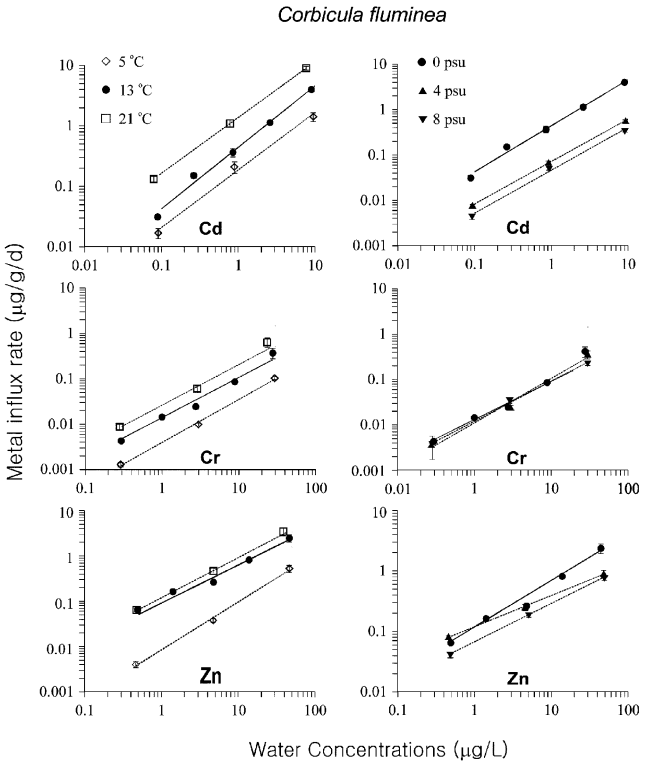

Fig. 3. Influx rates of Cd, Cr and Zn ($\mu g \cdot g^{-1}$ [dry wt] d^{-1}) into *Corbicula fluminea* from the dissolved phase at different concentrations ($\mu g \cdot g^{-1}$) under varying temperature (5, 13, 21°C) and salinity (0, 4, 8 psu) conditions. Values represent a mean (±SD) of 5 composite samples of 3 pooled clams. Log transformed metal influx rates (I_w) and metal concentrations in water (C_w) were linearly regressed as $I_w = k_u C_w^{\ b}$ (see Table 3 for k_u and b values for each relationship).

rate of all metals (Table 3). The k_u of Cr and Zn increased by ~5 X with the increase of temperature from 5 to 21°C. The k_u of Zn was lowest at the lowest temperature (5°C), increased by ~13 X at >13°C. Some salinity effects were observed most apparent for Cd uptake. The k_u of Cd at 4 and 8 psu were ~6 and ~10 X lower than those at 0 psu, respectively. The k_u of Cr seemed not to be influenced and that of Zn might be slightly influenced by salinity change only at 8 psu (Fig. 3; Table 3).

The amount of adsorbed metals on the shells increased proportionally with the water concentration (Fig. 4). Metal contents on the shells (M_{shell}) and metal concentrations in water (C_w) were regressed as $M_{shell} = a\ C_w^{\ b^*}$ (Table 3). Temperature significantly influenced on the amount of metals on shells. The effect of salinity on the metal adsorption on shells was not shown for Cr. However, the adsorbed Cd slightly decreased with the increase of salinity and the opposite trend was shown for Zn only at lower concentrations (Fig. 4).

Table 3. (A) Uptake rate constants (k_u) and regression coefficient (b) of dissolved metals in tissues of *C. fluminea*. (B) Constants (a) and sloes (b*) between dissolved metal concentration in water and adsorbed metals in shells of *C. fluminea* (see Fig. 3).

(A)

Temp. (°C)	Salinity (psu)	Cd		Cr		Zn	
		k_u	b	k_u	b	k_u	b
13	0	0.443	1.02	0.011	0.95	0.11	0.75
13	4	0.073	0.94	0.011	0.98	0.13	0.51
13	8	0.046	0.94	0.012	0.89	0.07	0.63
5	0	0.232	1.11	0.004	0.91	0.01	1.03
13	0	0.443	1.02	0.011	0.95	0.11	0.75
21	0	1.240	0.94	0.023	0.96	0.12	0.82

(B)

Temp. (°C)	Salinity (psu)	Cd		Cr		Zn	
		a	b*	a	b*	a	b*
13	0	5.68	1.07	1.57	1.03	1.27	1.29
13	4	5.11	0.98	1.71	1.04	3.87	1.05
13	8	3.48	0.97	1.70	1.02	2.52	1.10
5	0	1.17	1.24	0.73	1.00	1.08	1.50
13	0	5.68	1.07	1.57	1.03	1.27	1.29
21	0	6.15	1.01	1.71	0.83	1.94	1.06

Efflux rate of bioaccumulated metals

The metal accumulated during the 6-d exposure to the radiolabeled food and water was gradually lost from the tissue during the following 21-d depuration (Table 4). The release of metals was most rapid during the 2-d of the defecation period (data not shown). The release of metals following defecation represents the efflux from the tissue due to physiological turnover of assimilated metals. The physiological turnover of assimilated Cr and Zn followed the 2-compartmental model, of which each compartment can be explained by a first order exponential function (Table 4). The slopes (k_e) for Cr and Zn were changed around t = 5 d (7 d after the initiation of depuration). The first compartment contributed ~15% of whole assimilated Cr in clam tissues and 7~16% for Zn. The rate constant of loss for *C. fluminea* after t = 5 d increased in the order of Cd (0.0055-0.0057) < Zn (0.010-0.017) < Cr (0.019-0.023). Higher salinity might slow down the turnover rate of some metals in *C. fluminea*; the k_e of Cr and Zn at 8 psu were 80 and 60% of k_e in freshwater, respectively.

4. Discussion

Asiatic clam *C. fluminea* could accumulate Cd, Cr and Zn via both dissolved and particulate pathways. The characteristic of ingested particles significantly influenced the AEs of all elements. Physiological parameters related

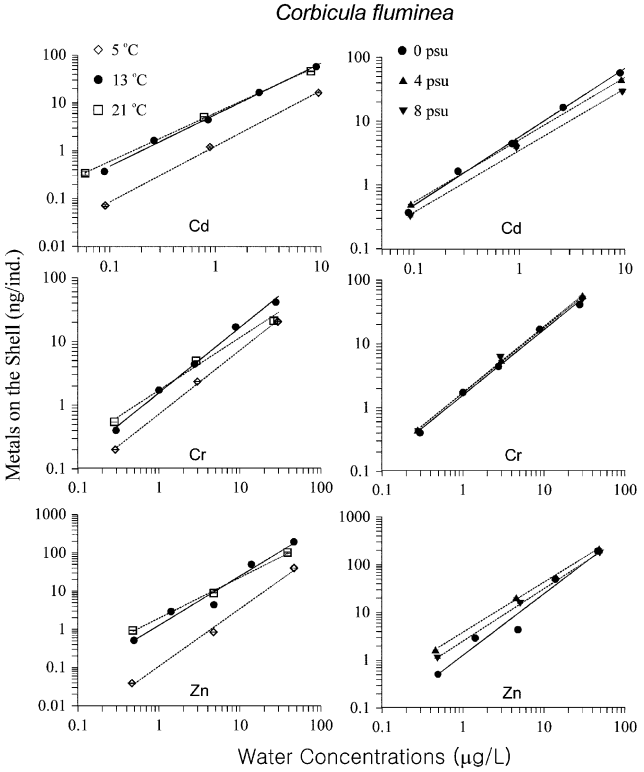

Fig. 4. Total contents of Cd, Cr and Zn adsorbed in the shell of *Corbicula fluminea* after the 6-hr exposure to radioactive media under varying temperature (5, 13, 21°C) and salinity (0, 4, 8 psu). Log transformed metal contents on the shells (M_{shell}) and metal concentrations in water (C_w) were linearly regressed as $M_{shell} = a\ C_w^{b*}$ (see Table 3 for a and b* values for each relationship).

to the metal bioaccumulation varied widely among elements and by external factors, such as salinity and temperature, which could vary the metal bioaccumulation in *C. fluminea* by altering uptake and the lost kinetics associated with metals as well as through the changing chemical speciation of metals. Using kinetic modeling in this study, we can evaluate how the variation in temperature and salinity can influence the metal bioaccumulation in *C. fluminea*.

Assimilation of ingested metals

The assimilation efficiencies of Cd associated with algae (70~90%) and sediment (40~65%) in *C. fluminea* were generally higher than the respective AEs in bivalves including cases involving various sediment-dwelling clams (*Macoma balthica, Potamocorbula amurensis* and *Ruditapes philippinarum*) and mussels (*Dreissena polymorpha, M. edulis* and *Perna viridis*) from previous studies (Roditi and Fisher 1999; Wang *et al.* 1996; Lee and Luoma 1998; Chong and Wang 2000; Ke and Wang 2001).

The inter-specific difference of metal AEs might be related to the species-specific digestive physiology and

Table 4. Elimination rate constants (k_e) of metals, ratio of each compartment in *Corbicula fluminea.*

	Temp. (°C)	Salinity (psu)	k_e	ratio (%)	period
Cd	13	0	0.0057	100	2~21 d
	13	8	0.0055	100	2~21 d
Cr	13	0	0.057	15	2~7 d
	13	0	0.023	85	7~21 d
	13	8	0.050	13	2~7 d
	13	8	0.019	87	7~21 d
Zn	13	0	0.055	16	2~7 d
	13	0	0.017	84	7~21 d
	13	8	0.022	7	2~7 d
	13	8	0.010	93	7~21 d

absorption rate of a metal across gut epithelium. *C. fluminea* might absorb Cd preferentially during the digestive procedure, since Cr(VI) and Zn AEs in *C. fluminea* were just comparable with those in other bivalves.

The AEs of metals could be influenced by food type as well as by external conditions such as salinity and temperature. Among various biotic and abiotic factors, the influence of the food type on metal AEs in *C. fluminea* was most apparent for all tested metals in this study. Consistently with the present study, Lee and Luoma (1998) showed that the increase in living cellular materials in the particulate assemblage were accompanied by increased AE in marine sediment dwelling clams, *M. balthica* and *P. amurensis*. The influence of food type on the metal AEs can be explained to some extent by subcellular partitioning of metals; the more metals in cytosolic form in the living cellular materials (Reinfelder and Fisher 1991). A relatively small fraction of metals was present in the form of cytosolic form in algal-poor suspended particles and sediments (Lee and Luoma 1998). Similarly, the nutritional quality of food might influence the AEs of metals as well as the AE of carbon in the food (Roditi and Fisher 1999).

The variation of salinity did not influence the assimilation of metals. Similarly, Blackmore and Wang (2003) reported that the AEs of green mussel *P. viridis* were not affected by salinity variations from 10 to 30 psu. Also, the influence of temperature on metal AE in *C. fluminea* was not apparent except in the case of Cd AEs where sediment varied considerably with temperature in the present study. The gut physiology might be influenced by temperature, which is related to the metabolic rate, including digestive enzyme activity. However, the effect of temperature was not consistent with metals and species in previous studies. The Ag AE in *Mytilus edulis* at lower temperatures (5°C) was 1.6-fold higher compared to 15°C (Wang and Fisher 1997). On the contrary, Hutchins *et al.* (1998) reported that AE of ^{241}Am in algae was 2.6 X greater at a higher

temperature (12°C compared to 2°C). A recently conducted Cd bioaccumulation study using freshwater insects *Chaoborus* showed that the influence of temperature on Cd AEs was not consistent among three species of the same genus (Croteau *et al.* 2002).

The significant relationship between AE and gut passage time of metals was shown when using data from freshwater treatments only. Consistently, the AEs of metals in *Mytilus edulis* and *Dreissena polymorpha*, had a significant relationship with gut passage time (Wang and Fisher 1996; Roditi and Fisher 1999; Selck *et al.* 1998). However, salinity sharply reduced the GPT of all metals associated with algal food, but the AEs of the metals did not respond to the change of GPT in this study. Consistent with our observation, Blackmore and Wang (2003) reported that the GPT of metals in *Perna viridis* decreased at higher salinity, but the metal AEs were not related to GPT. The increased salinity possibly influenced the digestive processes by reducing GPT and/or by altering the permeability of ions and water across gut epithelium. However, salinity varying from 2.5 to 25 psu influenced the Cd AE very little in terms of the estuarine amphipod (Schlekat *et al.* 1999). Metal AEs in green mussel *Perna viridis* were consistently higher in terms of salinity, which affected collected mussel preadapted to field conditions (Blackmore and Wang 2003). Therefore, the influence of salinity change on the metal AEs was related to the species-specific or even population-specific physiological processes as well as the element-specific processes.

The underlying mechanism for the relationship between salinity or temperature and metal uptake in the digestive system has not fully been understood. To predict metal bioaccumulation accurately using kinetic modeling, more endeavors should be incorporated to further elucidate the influence of salinity and temperature on metal assimilation by aquatic organisms.

Influx from the dissolved phase

Temperature has been estimated as a major environmental variable influencing metal bioaccumulation kinetic and the metabolic rate of animals (Phillips 1980). The increase of temperature may increase the bioavailability and toxicity of metals for testing animals (Fischer 1986; Odin *et al.* 1994; Rao and Khan 2000). The k_u of all Cd, Cr and Zn increased by 5.3 X, 5.8 X and 14.2 X, respectively, with the increase of temperature from 5 to 21°C in this study. The increase of k_u of metals was much higher than the increase of the metabolic rate predicted by Q_{10} law (3.2 X for the 16°C increase). Therefore, there might be more factors other than the metabolic rate affected by temperature variation. Water viscosity, which is decreased by temperature, could be a possible factor related to the temperature effect on metal uptake (Jørgensen *et al.* 1990; Podolsky 1994). The temperature might increase metal uptake rate by both increasing the metabolic rate and decreasing viscosity, which control the clearance rate of bivalves. Wang (2001) showed the significant relationship between the inter-specific clearance rates and metal uptake rates of various bivalves, while no significant relationship between the intra-specific clearance rates and metal uptake rates of each bivalve species observed in his study.

Many studies have evaluated the effect of salinity on the dissolved metal uptake (Fischer 1986; Nugegoda and Rainbow 1989*a, b*; Blust *et al.* 1992; Chan *et al.* 1992; Bjerregaard and Depledge 1994; Rainbow and Kwan 1995; Lee *et al.* 1998). Euryhaline organisms living at low salinities generally contained higher concentrations of metals than those living at higher salinities (Phillips 1980). The inverse relationship between the salinity and metal uptake rate has been explained by both geochemical and biological factors. Salinity can change the dissolved metal speciation, most importantly, the ratio of free ion metals, which are known as the most bioavailable (Mantoura 1978; Campbell 1995). The free ion activity of Cd and Zn generally decreased with the increase of Cl^- concentration in the solution. However, only the free ion model cannot account for the increase in metal uptake at lowered salinity levels. The major ion (*e.g.* Ca^{2+} and Mg^{2+}) concentration in the solution can also influence the Cd and Zn uptake by changing the permeability of the epithelial structures, competing for binding sites with the apical membrane surfaces, and decreasing metal transfer from the epithelium to the blood with increasing intracellular levels of calcium (Blust *et al.* 1992; Bjerregaard and Depledge 1994; Rainbow 1995). In contrast, the reduced salinity below the optimal range for marine animals can decrease ionic and osmotic fluxes between cells and surrounding media and also reduce the uptake of dissolved trace metals by impairing the clearance rate due to osmostic stress (Depledge 1990).

The influence of salinity on the influx rate was most apparent in the Cd influx in tissues of *C. fluminea*, but it was not consistent with elements in the present study. The 3~5 X decrease of free ion activity at higher salinity might partially contribute to the 6~10 X decrease of the dissolved Cd influx. The relatively small decrease of Zn uptake at 8 psu also might be related to the change of free ionic activity. Assuming that the Cr(VI) speciation was influenced minimally by salinity, the constant Cr uptake among different salinities may indicate that the filtration rate and permeability of gill epithelium of *C. fluminea* might not be affected by salinity (Rainbow and Kwan 1995). Therefore, the influence of salinity on the Cd and Zn influx rate might be explained by the combined effects of speciation change and increased binding competitions related to major ions.

Efflux of absorbed metals

The following defecation of unassimilated ingested materials, patterns of metal efflux could be described by a 1st order exponential decrease with one (Cd) or two compartments (Cr and Zn) in *C. fluminea* from both freshwater and saltwater treatments (8 psu). Multi-compartmental uptake and loss models, typically including fast exchanging pool and slower exchanging pool(s), were well illustrated by Nugegoda and Rainbow (1989*b*) and employed in many other studies (Dahlgaard 1986; Wang *et al.* 1996; Wang *et al.* 1997; Roditi and Fisher 1999). The multi-compartments may reflect the distribution kinetics between various organs or the subcellular partitioning. However, the loss pattern of each element will depend on metal-specific or species-specific physiological conditions, as well as the exposure duration, external condition, and exposure route (Wang *et al.* 1996; Roditi and Fisher 1999).

The efflux rate constants (k_e) for Cd in *C. fluminea* (0.0055~0.0057) were not influenced by salinity and were relatively low compared to those in other freshwater and saltwater bivalves including *M. edulis*, *M. balthica*, *P. amurensis* and *D. viridis* (0.011~0.021) (Wang *et al.* 1996; Lee and Luoma 1998; Roditi and Fisher 1999). Similarly, in a 30-d depuration experiment, Inza *et al.* (1998) found no significant efflux of Cd from *C. fluminea* tissues except Cd in gill, which decreased only 22% for that period.

The effect of salinity on the metal efflux rate of *C. fluminea* was most apparent in Zn followed by Cr in this study. The lower efflux rate in higher salinity might reflect the osmotic regulation involving freshwater clam, *C. fluminea* to maintain the composition of the internal environment within certain physiological limits (Blust *et al.* 1992). Acclimation to higher salinity may involve the alteration of the exchange surfaces (*e.g.* gill and gut epithelium) and these physiological alterations may influence the movement of water and ions across the exchange surfaces. Animals increasing the permeability of water and ions between internal and external media across gill or gut epithelium at the higher salinity and urine production might decrease at higher salinities due to the physiological responses to managing osmotic balance (Nugegoda and Rainbow 1989*b*). It is consistent with the result that showed that *C. fluminea* at higher salinity had a lower efflux rate of Cr and Zn in the present study, because metals can be released as salt in urine.

5. Conclusion

We have demonstrated that *C. fluminea* could absorb Cd, Cr and Zn from both dietary and dissolved sources under varying salinity and temperature conditions. The various physiological parameters affecting metal bioaccumulation in *C. fluminea* exposed to the environmentally realistic contamination were determined in the laboratory.

The variation in temperature and salinity could influence the major physiological parameters in the toxicokinetic model, describing metal bioaccumulation in aquatic organisms; the metal influx rate from water/food, and also the metal efflux rate from tissues. Those parameters can be used to understand and predict metal bioaccumulation in natural conditions. Therefore, the most accurate bioaccumulation models should incorporate variations in the various physiological rates over the environmentally realistic range of those environmental factors.

The rate of uptake and loss of metals in food and water media were influenced by various external factors such as food particle composition, salinity, and temperature as well as the metal concentration in solutions. The effect of temperature and salinity on metal kinetic parameters was most apparent in the dissolved uptake rate of metals, while most metal AEs were relatively constant over the experimental range of temperature and salinity.

Dissolved metal uptake increased with temperature. However, the influence of salinity was not consistent among metals. The dissolved Cd influx rate was most influenced by salinity change, partially due to the chemical speciation change. The salinity influenced the metal accumulation in *C. fluminea* by influencing both the influx and efflux rate of metals in this study. The efflux rate of Zn was considerably decreased at higher salinity, resulting in the higher predicted concentrations of Zn in *C. fluminea* from the model estimation, although the influx of dissolved Zn was slower under the higher salinity treatment conditions.

The relatively high Cd accumulation capacity of *C. fluminea*, characterized by high AE, a high dissolved influx rate, and a low efflux rate of Cd, suggested that this clam species can be used as an efficient biomonitor for the contamination of metals in freshwater and estuarine environments, especially for Cd.

References

Bjerregaard, P. and M.H. Depledge. 1994. Cadmium accumulation in *Littorina littorea*, *Mytilus edulis* and *Carcinus maenas*: The influence of salinity and calcium ion concentrations. *Mar. Biol.*, **119**, 385-395.

Blackmore, G. and W.-X. Wang. 2003. Inter-population differences in Cd, Cr, Se and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. *Aquat. Toxicol.*, **62**, 205-218.

Blust, R., E. Kochelbert, and M. Baillieul. 1992. Effect of salinity on the uptake of cadmium by the brine shrimp *Artemia franciscana*. *Mar. Ecol. Prog. Ser.*, **84**, 245-254.

Campbell, P.G.C. 1995. Interaction between trace metals and aquatic organisms: A critique of the free-ion activity model. p. 45-102. In: *Metal speciation and aquatic systems.* ed. by

A. Tessier and D.R. Turner. John Wiley & Sons Ltd., New York.

Chan, H.M., P. Bjerregaard, P.S. Rainbow, and M.H. Depledge. 1992. Uptake of zinc and cadmium by two different populations of shore crab *Carcinus maenus* at different salinity. *Mar. Ecol. Prog. Ser.*, **86**, 91-97.

Chong, I. and W.X. Wang. 2000. Assimilation of Cd, Cr, and Zn by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. *Environ. Toxicol. Chem.*, **19**, 1660-1667.

Croteau, M.-N., L. Hare, and A. Tessier. 2002. Influence of temperature on Cd accumulation by species of the biomonitor *Chaoborus*. *Limnol. Oceanogr.*, **47**, 505-514.

Dahlgaard, H. 1986. Effects of season and temperature on long-term *in situ* loss rates of Pu, Am, Np, Eu, Ce, Ag, Tc, Zn, Co, and Mn in a Baltic *Mytilus edulis* population. *Mar. Ecol. Prog. Ser.*, **33**, 157-165.

Decho, A.W. and S.L. Luoma. 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma Balthica*: Significance to the absorption of carbon and chromium. *Mar. Ecol. Prog. Ser.*, **78**, 303-314.

Depledge, M.H. 1990. Models of regulation and accumulation of trace metals in marine invertebrates. *Comp. Biochem. Physiol.*, **97c**, 1-7.

Fischer, H. 1986. Influence of temperature, salinity, and oxygen on the cadmium balance of mussels *Mytilus edulis*. *Mar. Ecol. Prog. Ser.*, **32**, 265-278.

Folk, R.L. 1954. The distribution between grain size and mineral composition in sedimentary rock nomenclature. *J. Geol.*, **23**, 1-12.

Goldberg, E.D., M. Koide, V. Hodge, A.R. Flegal, and J. Martin. 1983. US Mussel Watch: 1977-1978 results on trace metals and radionuclides. *Estuar. Coast. Shelf. Sci.*, **16**, 69-93.

Graney, R.L., D.S. Cherry, and J. Cairns, Jr. 1983. Heavy metal indicator potential of the asiatic clam (*Corbicula fluminea*) in artificial stream systems. *Hydrobiologia*, **105**, 81-88.

Hutchins, D.A., I. Stupakoff, S. Hook, S.N. Luoma, and N.S. Fisher. 1998. Effects of arctic temperatures on distribution and retention of the nuclear waste radionuclides ^{241}Am, ^{57}Co, and ^{137}Cs in bioindicator bivalve *Macoma balthica*. *Mar. Environ. Res.*, **45**, 17-28.

Inza, B., F. Ribeyre, and A. Boudou. 1998. Dynamics of cadmium and mercury compounds (inorganic mercury or methylmercury): Uptake ad depuration in *Corbicula fluminea*. Effects of temperature and pH. *Aquat. Toxicol.*, **43**, 273-285.

Jørgensen, C.B., R.S. Larsen, and H.U. Riisgård. 1990. Effects of temperature on the mussel pump. *Mar. Ecol. Prog. Ser.*, **64**, 89-97.

Ke, C. and W.-X. Wang. 2001. Bioaccumulation of Cd, Se, and Zn in an esturine oyster (*Crassostrea rivularis*) and a coastal oyster (*Saccostrea glomerata*). *Aquat. Toxicol.*, **56**, 33-51.

Langston, W.J. and S.K. Spence. 1995. Biological factors involved in metal concentrations observed in aquatic organisms. p. 407-478. In: *Metal speciation and bioavailability in aquatic systems*. ed. by A. Tessier. and D.R. Turner. John Wiley & Sons, Chichester.

Lee, B.G. and S.N. Luoma. 1998. Influence of microalgal biomass on absorption efficiency of Cd, Cr and Zn by two bivalves from San Francisco Bay. *Limnol. Oceanogr.*, **43**, 1455-1466.

Lee, B.G., W.G. Wallas, and S.N. Luoma. 1998. Uptake and loss kinetics of Cd, Cr and Zn in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: Effects of size and salinity. *Mar. Ecol. Prog. Ser.*, **175**, 177-189.

Lee, B.G., S. Griscom, J.S. Lee, H.J. Choi, C.-H. Koh, S.N. Luoma, and N.S. Fisher. 2000. Influence of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments. *Science*, **287**, 282-284.

Lee, J.S., B.G. Lee, H. Yoo, C.-H. Koh, and S.N. Luoma. 2001. Influence of reactive sulfide (AVS) and supplementary food on Ag, Cd and Zn bioaccumulation in the marine polychaete, *Neanthes arenaceodentata*. *Mar. Ecol. Prog. Ser.*, **216**, 129-140.

Luoma, S.N., C. Johns, N.S. Fisher, N.A. Steinberg, R.S. Oremland, and J.R. Reinfelder. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. *Environ. Sci. Technol.*, **26**, 485-491.

Mantoura, R.F.C., A. Dickson, and J.P. Riley. 1978. The complexation of metals with humic materials in natural waters. *Estuar. Coast. Mar. Sci.*, **6**, 387-408.

Mishima, J. and E.P. Odum. 1963. Excretion rate of Zn^{65} by Littorina irrorata in relation to temperature and body size. *Limnol. Oceanogr.*, **8**, 39-44.

Morel, F.M.M., J.C. Westall, J.G. Rueter, and J.P. Chaplick. 1975. Description of the algal growth media Aquil and Fraquil. Department of Civil Engineering, Massachusetts Institute of Technology, Technical note No.16, Cambridge, MA., USA.

Nugegoda, D. and P.S. Rainbow. 1989*a*. Effects of salinity changes on zinc uptake and regulation by the decapod crustaceans *Palaemon elegans* and *Palaemonetes varians*. *Mar. Ecol. Prog. Ser.*, **51**, 57-75.

Nugegoda, D. and P.S. Rainbow. 1989*b*. Salinity, osmolarity, and zinc uptake in *Palaemon elegans* (Crustacea: Decapoda). *Mar. Ecol. Prog. Ser.*, **55**, 149-157.

Odin, M., F. Ribeyre, and A. Boudou. 1994. Temperature and pH effects on cadmium and methylmercury bioaccumulation by nymphs of the burrowing mayfly Hexagenia regida, from water column or sediment source. *Arch. Environ. Contam. Toxicol.*, **31**, 339-349.

Phillips, D.J.H. 1980. Quantitative aquatic biological indicators: Their use to monitor trace metal and organochlorine pollution. Applied Science Publishers Ltd., London.

Podolsky, R.D. 1994. Temperature and water viscosity: physiological versus mechanical effects on suspension feeding. *Science*, **265**, 100-103.

Rainbow, P.S. 1995. Physiology, physicochemistry and metal uptake-a crustacean perspective. *Mar. Pollut. Bull.*, **31**, 55-59.

Rainbow, P.S. and M.H. Kwan. 1995. Physiological responses and the uptake of cadmium and zinc by the amphipod crustacean *Orchestia gammarellus*. *Mar. Ecol. Prog. Ser.*, **127**, 87-102.

Rao, D.G.V.P. and M.A.Q. Khan. 2000. Enhancement of copper toxicity by high temperature and its relationship with respiration and metabolism. *Wat. Environ. Res.*, **72**, 175-178.

Reinfelder, J.R. and N.S. Fisher. 1991. The assimilation of elements ingested by marine copepods. *Science*, **251**, 794-796.

Roditi, H.A. and N.S. Fisher. 1999. Rates and routes of trace element uptake in zebra mussels. *Limnol. Oceanogr.*, **44**, 1730-1749.

Schlekat, C.E., A.W. Decho, and G.T. Chandler. 1999. Dietary assimilation of cadmium associated with bacterial exopolymer sediment coastings by the estuarine amphipod *Leptocheirus plumulosus*: Effects of Cd concentration and salinity. *Mar. Ecol. Prog. Ser.*, **183**, 205-216.

Selck, H., V.E. Forbes, and T.L. Forbes. 1998. Toxicity and toxicokinetics of cadmium in *Capitella* sp. I: Relative importance of water and sediment as routes of cadmium uptake. *Mar. Ecol. Prog. Ser.*, **164**, 167-178.

Wang, W.-X., N.S. Fisher, and S.N. Luoma. 1995. Assimilation of trace elements ingested by the mussel *Mytilus edulis*: Effects of algal food abundance. *Mar. Ecol. Prog. Ser.*, **129**, 165-176.

Wang, W.-X. and N.S. Fisher. 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: Effects of food composition. *Limnol. Oceanogr.*, **41**, 197-207.

Wang, W.-X., N.S. Fisher, and S.N. Luoma. 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.*, **140**, 91-113.

Wang, W.-X. and N.S. Fisher. 1997. Modeling metal bioavailability for Marine Mussels. *Rev. Environ. Contam. Toxicol.*, **151**, 39-65.

Wang, W.-X., S.B. Griscom, and N.S. Fisher. 1997. Bioavailability of Cr(III) and Cr(VI) to marine mussels from solute and particulate pathways. *Environ. Sci. Technol.*, **31**, 603-611.

Wang, W.-X. 2001. Comparison of metal uptake rate and absorption efficiency in marine bivalves. *Environ. Toxicol. Chem.*, **20**, 1367-1373.